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Modulating Wnt Signaling Pathway to Enhance Allograft Integration in Orthopedic Trauma Treatment

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14. ABSTRACT
The research project was designed to test a novel approach of modulating Wnt signaling pathway in the bone tissue repair by using monoclonal antibodies against sclerostin (Sost) and DKK-1 (donated by Amgen Inc., Thousand Oaks, CA under MTA). Since the previous annual report, the project has progressed at a rapid pace. We resolved all initial technical difficulties and successfully completed all the surgical procedures, harvested samples at prescribe time points and evaluated new bone formation at the allograft site using µCT scans and partially completed mechanical testing. Data presented in report reveals statistically that use of anti-Sost or anti-Dkk-1 antibodies enhances new bone formation around the allograft over all time points. Anti-Dkk-1 antibody treatment also seems to be superior to anti-Sost treatment. Mechanical testing show increasing strength over time. We are in the process of finalizing the mechanical testing and histology. Data so far supports our hypothesis.

15. SUBJECT TERMS
Slow progress in data analyses.

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Introduction

The scope of this project is to evaluate if the use of a novel anabolic treatment that targets the specific signaling pathways during osteogenesis that promotes bone healing will enhance the integration of allografts to the host bone in an animal model that simulates severe bone loss due to local trauma. In general, it is known that several different growth factors aid bone regeneration. In previous studies we have reported enhanced bone regeneration when growth factors, such as bone morphogenetic protein (BMP), are applied directly at the site of injury (1-10). It is also known that mechanical stimuli at the regenerate also accelerate the healing process. We and others have demonstrated that pulses of low intensity ultrasound, delivering mechanical stimulus, accelerates fracture healing (11-14). However, the focus of the proposed application is to employ a novel approach of modulating the LRP5/Wnt cell signaling pathway which is known to be critically involved in osteogenesis in order to repair large bone defects such as those experienced by soldiers in the battlefield due to ballistics related trauma to the extremities. Monoclonal antibodies raised against sclerostin and dickkopf-1 (Dkk-1) were proposed to be the test reagents employed to modulate the Wnt signaling pathway. An agreement with Amgen Inc. (Thousand Oaks, CA) was established for them to donate the reagents.

In order to carry out this research, we had proposed an animal model of segmental bone defect in the rat femur. In previouas research projects in our laboratory we have employed this model to study the efficacy of combining BMP-2 and low intensity pulsed ultrasound to improve new bone regeneration in the gap. In the current study we had proposed to place an allograft in the created gap and to then treat the animals with systemic delivery of anti-sclerostin or anti-Dkk-1 antibodies for the prescribed period of time. The endpoints proposed were x-ray and μCT imaging, mechanical testing and histology.

In addition to the text above (similar to last annual report), we have now completed the most time consuming portion of the project. All the surgeries, systemic treatments, sample harvesting, μCT scanning and evaluation has been completed. Majority of the mechanical testing has also been completed. Overall, we have completed approximately 90% of the tasks from the SOW. With the new end date of March 29, 2014, we will report our overall findings in the final report that is due April 29, 2014.

Body

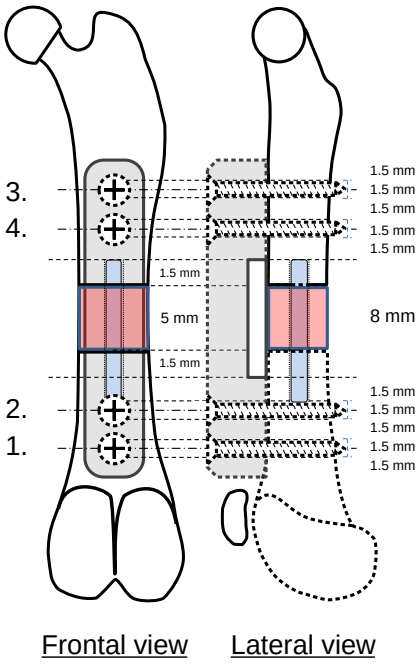
We hypothesized that **neutralizing the LRP5/Wnt pathway inhibitors Sost or DKK1 with monoclonal antibodies will enhance allograft integration to the host bone.** The proposed work in this project was designed to test this hypothesis by addressing two specific aims.

Aim 1: Determine the effect of modulating the LRP-5/Wnt pathway with anti-Sost monoclonal antibody on allograft incorporation in a rat segmental repair model using radiographical, morphological and mechanical endpoints.

Aim 2: Determine the effect of modulating the LRP-5/Wnt pathway with anti-Dkk1 monoclonal antibody on allograft incorporation in a rat segmental repair model using radiographical, morphological and mechanical endpoints.

Within each these aims we proposed to use fresh frozen and freeze-dried allografts to emulate clinical scenarios where banked tissue available for use in patients is processed by these procedures.

Figures 1 and 2 depict our modified and working surgical model to ensure perfect placement of allograft that not only stays in place but also stays aligned with the host bone. This approach was critical in making sure that results from the study would be consistent and reliable.



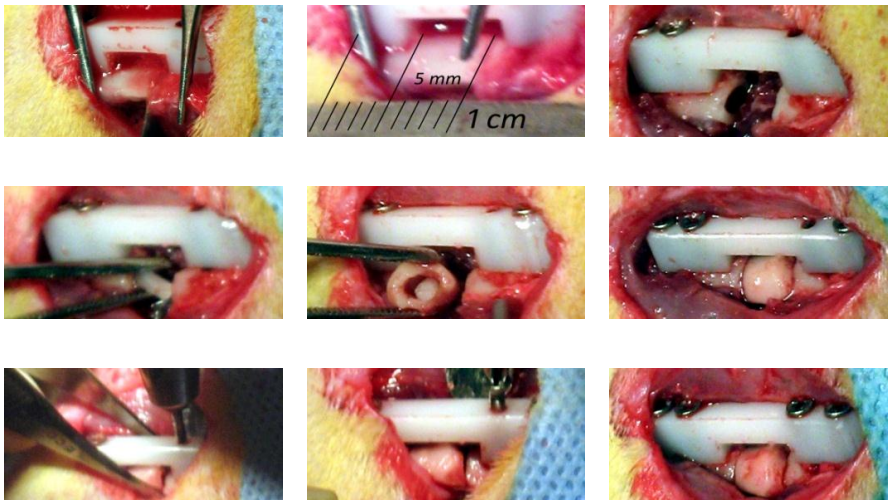


Figure 2: Surgical steps (top left to bottom right) for allograft placement in the segmental defect. Note the internal fixator (white) and relative location of the allograft.

Figure 1: Schematic representation of the allograft (pink) placement in the segmental defect. Note that the allograft is held in place, and thus stays aligned with host bone, using a polyethylene wire (blue). The whole defect is stabilized with an internal fixator (gray) that is secured with four screws.

Using this approach, we have completed all proposed surgical procedures. All groups have been treated with respective treatments for the stated duration. In vivo radiographs as well ex-vivo radiographs at the time of harvesting have also been completed for all samples.

We have analyzed all harvested bones from Fresh Frozen and Freeze-Dried allografts at 4, 8 and 12 week time points for saline, anti-Sost and anti-Dkk1 using µCT scanning. Data is presented below. Quantitative output provides an extensive set of data but we have chosen to present the most relevant parameters that are reflected in the following outcomes.

Total Volume (TV) – this indicates the overall hard callus volume around the allograft and is suggestive of earlier healing events.

Bone volume (BV) – this indicates the amount of new bone formed around the allograft and represents the overall quantity and rate of bone regeneration. In general, higher BV correlates with better mechanical competence.

Bone Volume over Total Volume (BV/TV) – this outcome indicates the porosity of the new bone around the allograft.

Bone Mineral Content – this outcome indicates how much mineral is present in the healing area and correlates with bone density.

Pictures below (**Figures 3, 4 and 5**) show radiograph and µCT images for representative samples from each group (Same as last annual report).

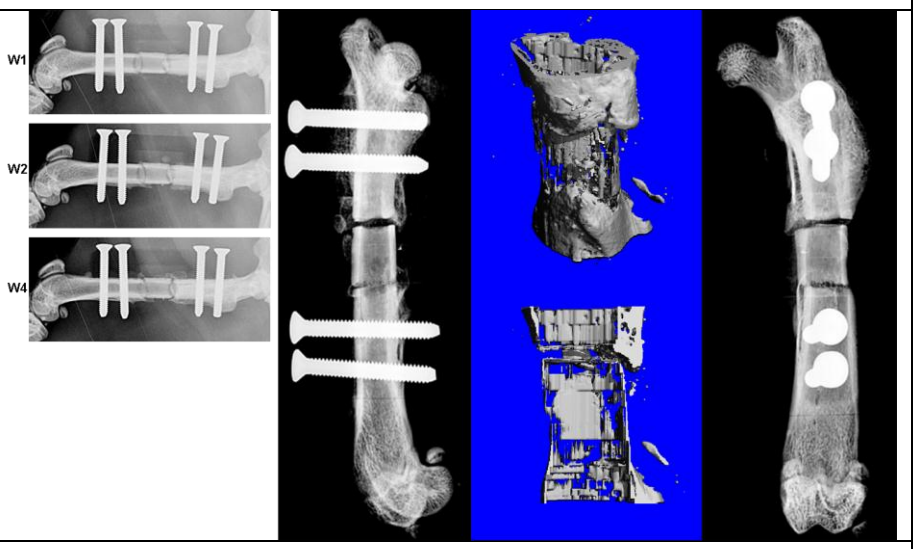
Figure 3: In vivo radiographs, ex vivo radiographs and µCT scans for 4 week time point

Saline treatment

Left to right:
1 – in vivo radiographs
2 – ex vivo radiograph (AP)
3 - µCT showing new bone only
4 – ex vivo radiograph (Lateral)

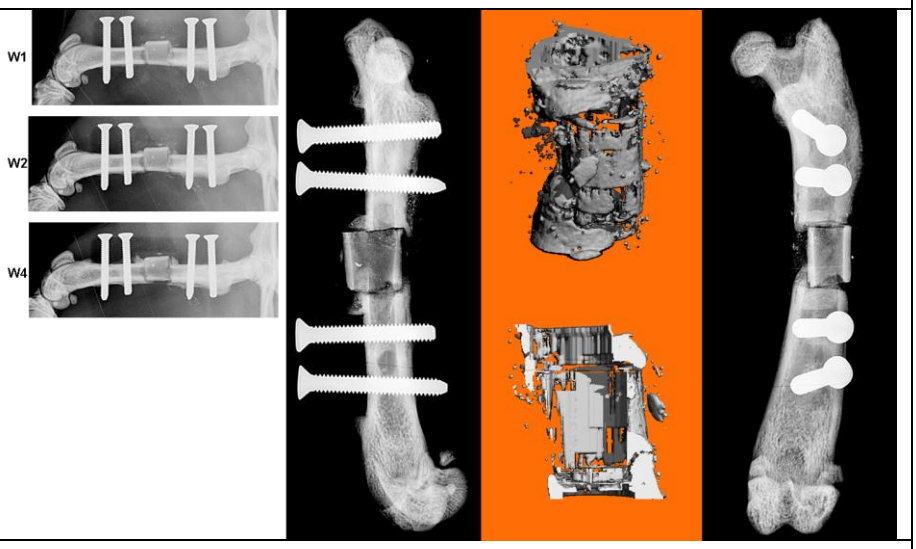
W1, W2 etc. refer to the week at which the in vivo radiograph was taken.

Same layout for all panels in Figures 3, 4 and 5.



Anti-Sost antibody Treatment

Layout as described above.



Anti-Dkk1 antibody Treatment

Layout as described above.

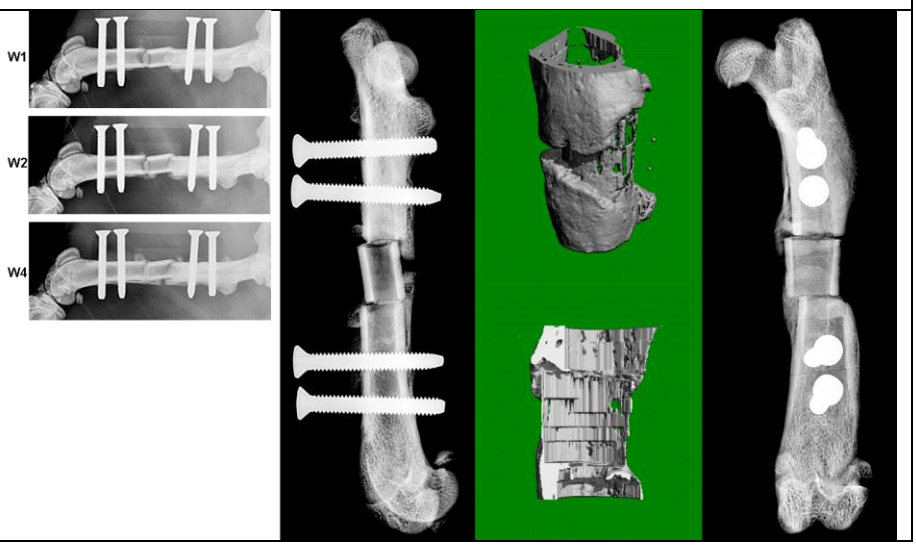
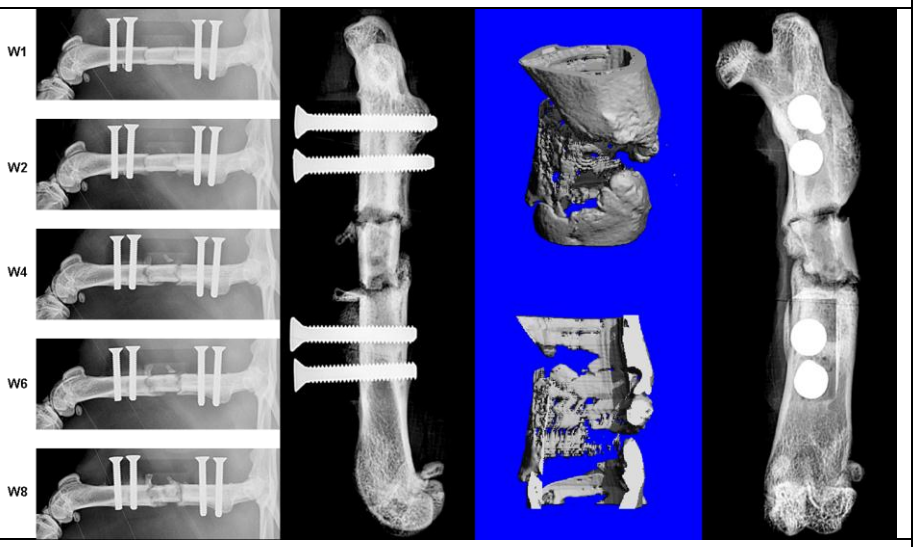


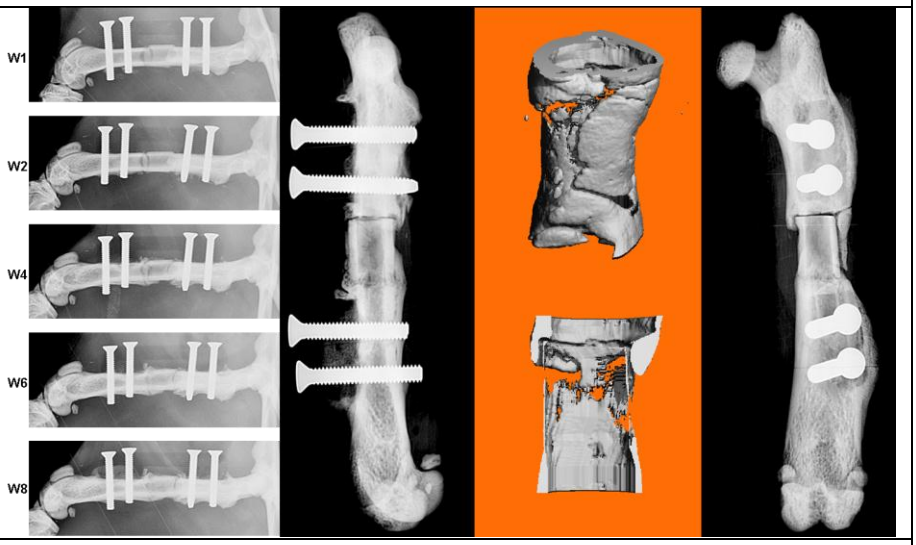
Figure 4: In vivo radiographs, ex vivo radiographs and μCT scans for 8 week time point

Saline treatment

Please see Figure 3 for layout description.



Anti-Sost antibody Treatment



Anti-Dkk1 antibody Treatment

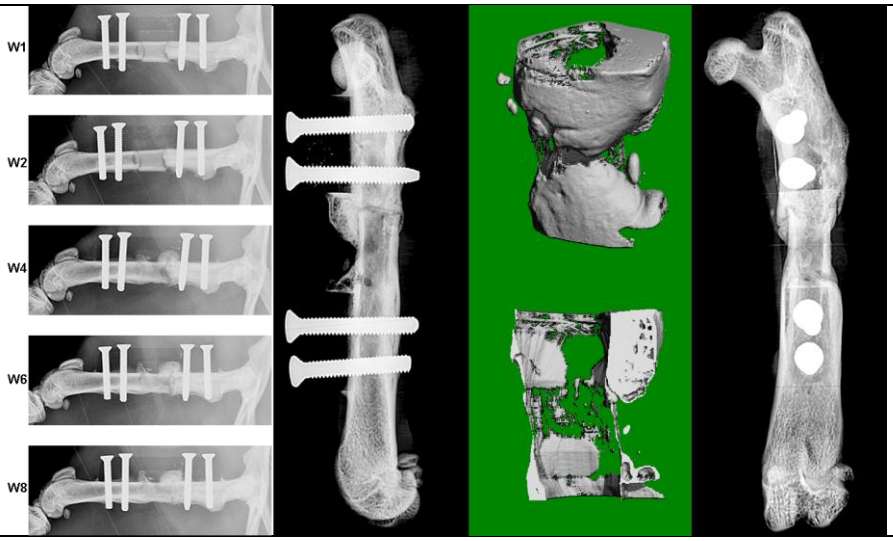
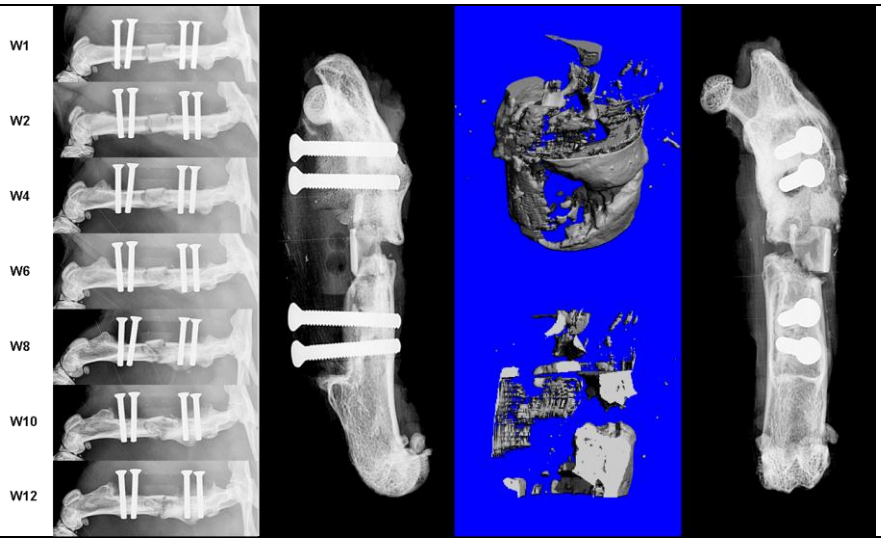


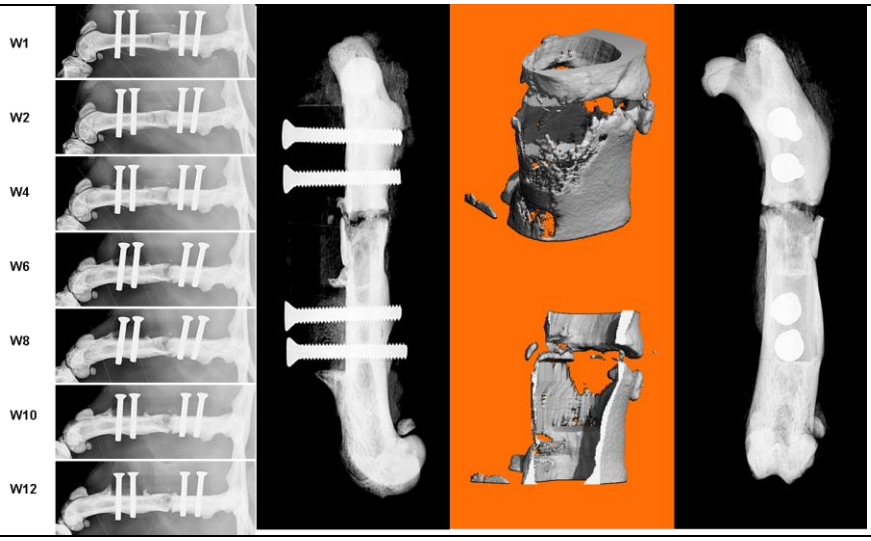
Figure 5: In vivo radiographs, ex vivo radiographs and μCT scans for 12 week time point

Saline treatment

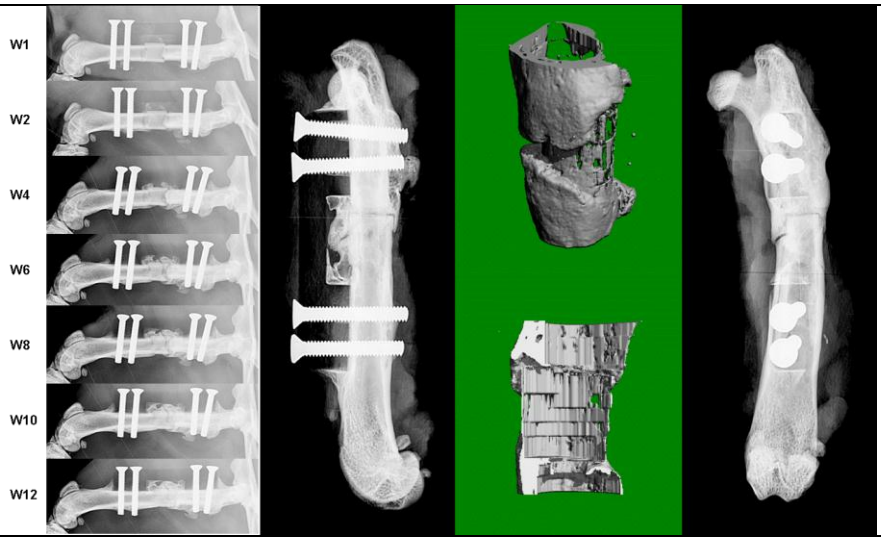
Please see Figure 3 for layout description.



Anti-Sost antibody Treatment



Anti-Dkk1 antibody Treatment



µCT evaluation data was analyzed for four most relevant outcomes as stated above. The data from Fresh Frozen and Freeze-Dried allografts are presented separately. Table 1 shows the number of samples analyzed for each graft, time point and treatment.

Table 1: Sample numbers analyzed for each treatment group.

Fresh Frozen	Saline Treatment	Anti-Sost Treatment	Anti-Dkk1 Treatment
4 week	16	16	16
8 weeks	16	16	14
12 weeks	16	11	15
Freeze Dried	Saline Treatment	Anti-Sost Treatment	Anti-Dkk1 Treatment
4 week	15	14	15
8 weeks	13	14	16
12 weeks	14	14	15

Figures 6 and 7 depict graphs of µCT quantitative data for all treatments and time points from Fresh Frozen allografts. The data is presented as a function of time (Figure 6; 4 weeks, 8 weeks, 12 weeks) as well as function of treatment (Figure 7; Saline, Scl-Ab, Dkk1-Ab). Statistical analysis was performed on all groups and comparisons showing significance at $p<0.05$ is shown on the graphs.

In general, the data reveals that both anti-Sost and anti-Dkk1 antibody treatments enhanced bone formation (indicated by increase in BV, BV/TV and BMC) when compared with saline treatment. The mechanical testing has not been completed for all the samples but we expect that it will reflect the findings from the µCT data. If proven true, this would represent a practical means of enhancing repair of large bone defects in orthopedic trauma and can be translated into clinical practice in the near future.

Figures 8 and 9 depict graphs of µCT quantitative data for all treatments and time points from Freeze Dried allografts. The data is presented as a function of time (Figure 6; 4 weeks, 8 weeks, 12 weeks) as well as function of treatment (Figure 7; Saline, Scl-Ab, Dkk1-Ab). Statistical analysis was performed on all groups and comparisons showing significance at $p<0.05$ is shown on the graphs. Overall findings were similar to the Fresh Frozen allografts.

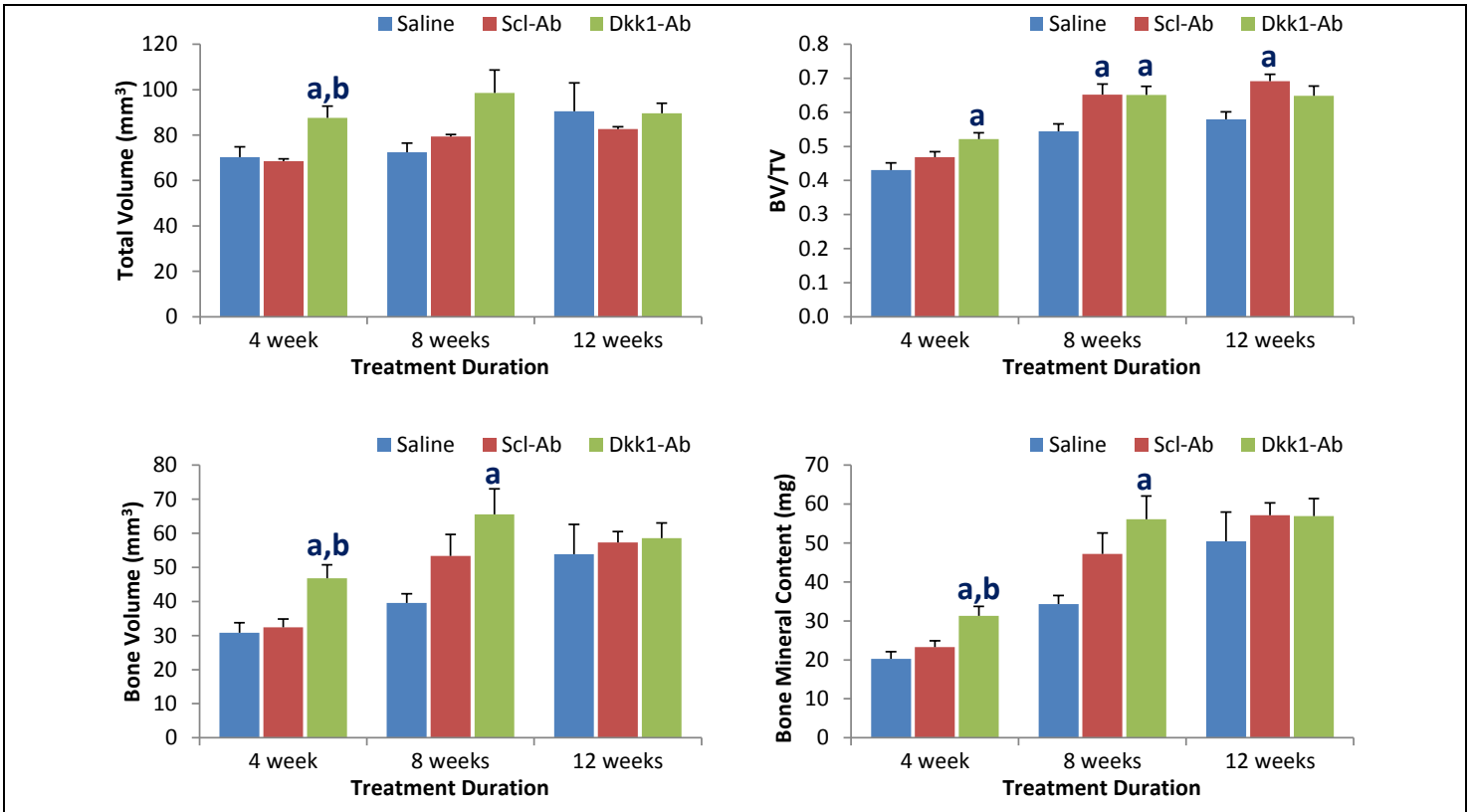


Figure 6: µCT data for Fresh Frozen allografts as a function of treatment duration.
The bars are color coded for easier interpretation. Blue = Saline, Red = anti-Sost, Green = anti-Dkk1.
a = $p<0.05$ relative to Saline, b = $p<0.05$ relative to Scl-Ab.

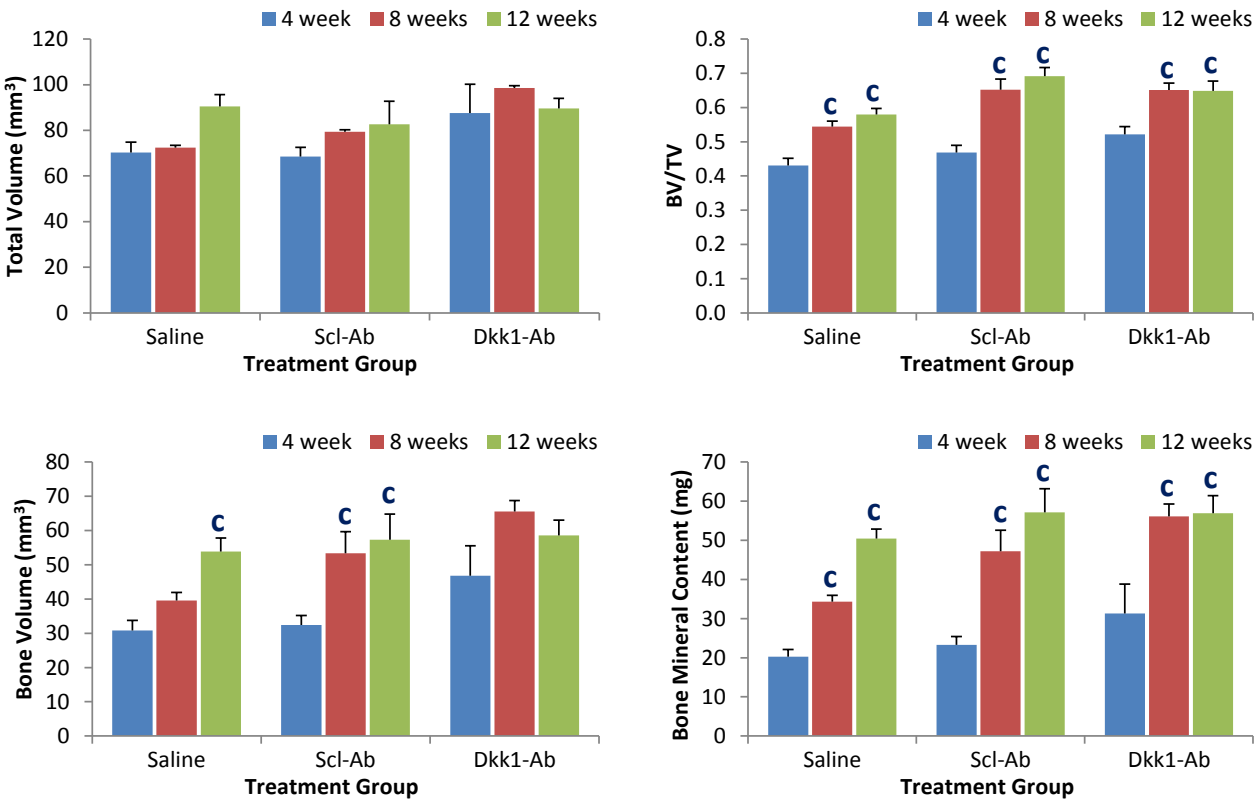


Figure 7: µCT data for Fresh Frozen allografts as a function of systemic treatment.
The bars are color coded for easier interpretation. Blue = 4 weeks, Red = 8 weeks, Green = 12 weeks.
c = $p<0.05$ relative to 4 Weeks, d = $p<0.05$ relative to 8 weeks.

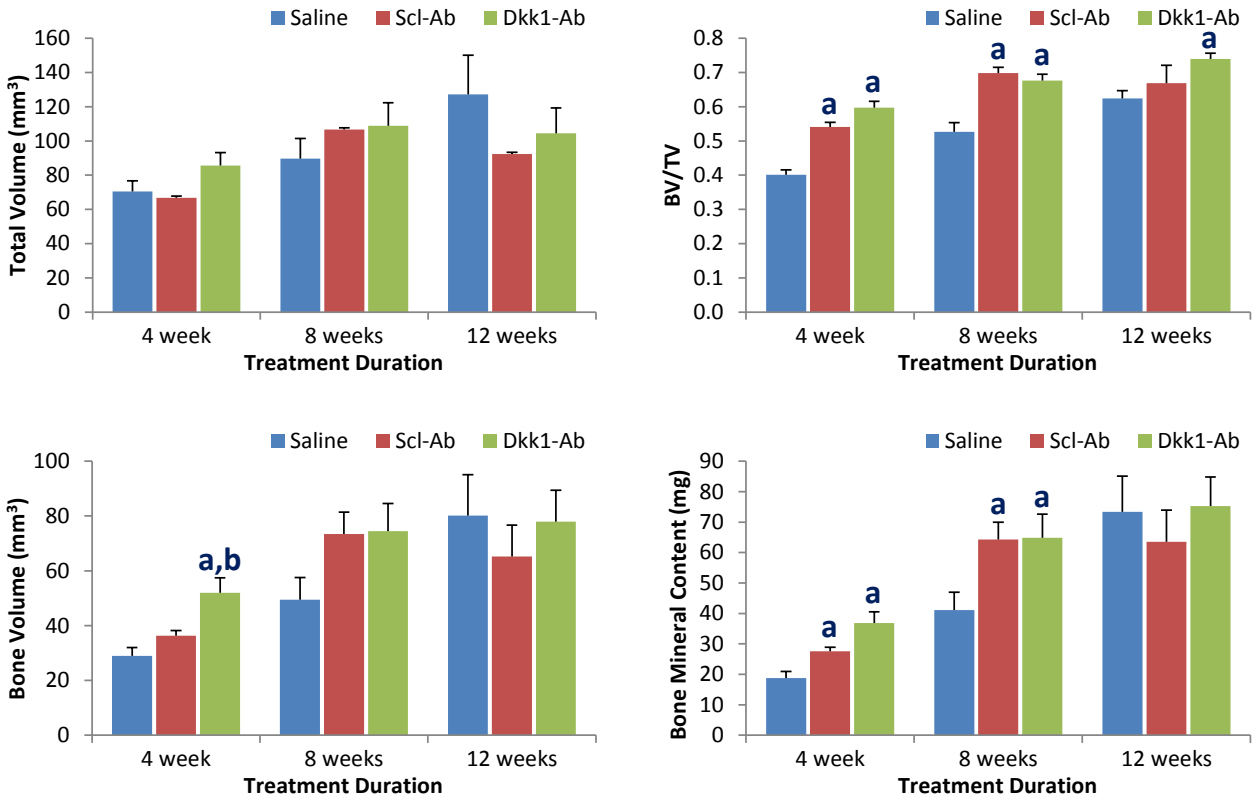


Figure 8: µCT data for Freeze Dried allografts as a function of treatment duration.
The bars are color coded for easier interpretation. Blue = Saline, Red = anti-Sost, Green = anti-Dkk1.
a = $p<0.05$ relative to Saline, b = $p<0.05$ relative to Scl-Ab.

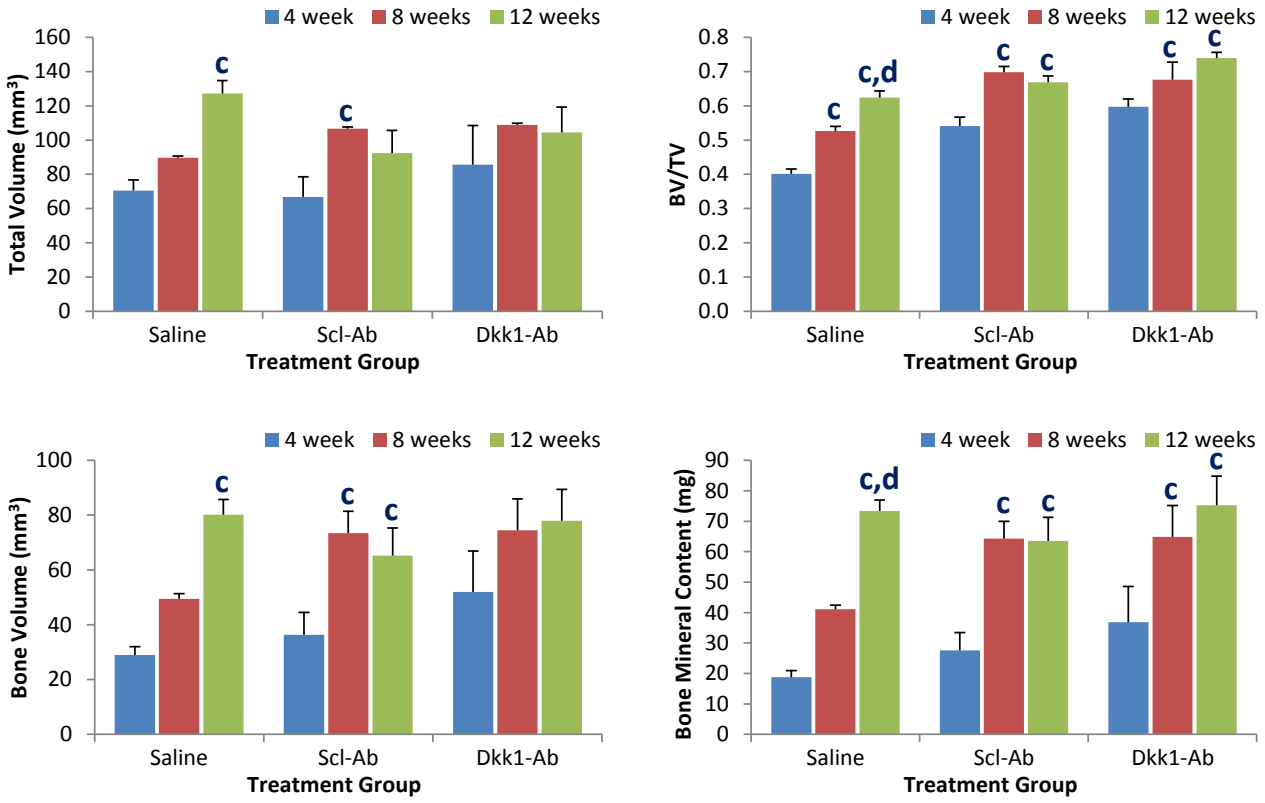


Figure 9: µCT data for Freeze Dried allografts as a function of systemic treatment.
The bars are color coded for easier interpretation. Blue = 4 weeks, Red = 8 weeks, Green = 12 weeks.
c = $p<0.05$ relative to 4 Weeks, d = $p<0.05$ relative to 8 weeks.

Figure 10 depicts a subset of the mechanical torsional test data in Fresh Frozen allografts. It is interesting to see that all treatments (including Saline) show time dependent increase in torsional strength (right and panel) but Dkk1 treatment is exhibiting the greatest response. This observation is unexpected and is worth following in the future.

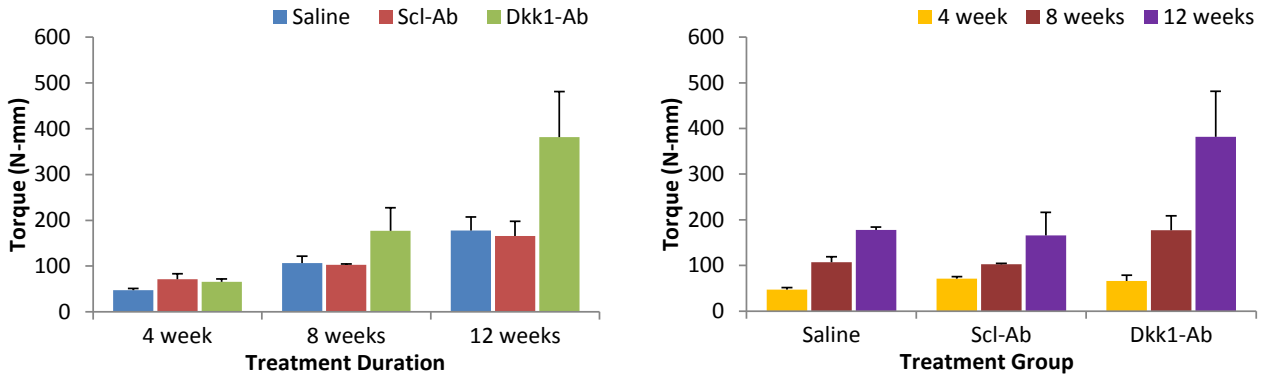


Figure 10: Mechanical data for Fresh Frozen allograft.
The bars are color coded for easier interpretation. Blue = Saline, Red = Scl-Ab, Green =. Dkk1-Ab

Orange = 4 weeks, Dark Brown = 8 weeks, Purple = 12 weeks.

Key Research Accomplishments

- All surgical procedures have been completed.
- All systemic treatments have been completed.
- All in vivo and ex vivo radiographs have been completed.
- All treated bones have been harvested.
- All harvested samples have been scanned by μCT and evaluated for multiple parameters.
- Most samples have been mechanically tested and data extracted for multiple parameters.
- Histological evaluation of subset of samples is underway.

Reportable Outcomes

- None at this stage.

Conclusion

- The observations made based on μCT and mechanical data available at this stage indicate that modulating the LRP/Wnt signaling pathway with anti-Sost and anti-Dkk1 monoclonal antibodies enhances new bone formation around allografts in a rat segmental defect model.

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Appendices

- None